REMARKS

Prior to filing the RCE, claims 11-33 were pending. By this amendment, applicants have amended claims 15-18 and 22-27, cancelled claims 11-14, 19-20 and 28-37, and added new claims 38-41. Accordingly claims 15-18, 21-27, and 38-41 are currently pending.

Applicants wish to thank Examiner Foley for the courtesy of the telephone interview on June 14, 2002 with Algis Anilionis. Applicants also wish to thank Examiner Foley and Examiner Housel for the courtesy of the personal interview at the Patent and Trademark Office on February 26, 2003 with Dr. Anilionis.

The undersigned is currently prosecuting this case. Applicants wish to thank Examiner Foley for the courtesy of the telephone interview on May 13, 2003 with the undersigned. During the telephone interview, applicants discussed with the examiner new prior art references, whether to file an RCE to make the new prior art references of record, and proposed claims. During the discussion, it was decided that applicants would file an RCE. Applicants stated that a Preliminary Amendment responding to the final Office Action of June 4, 2002 would be filed along with the RCE. In addition, applicants offered to provide in the Preliminary Amendment a synopsis of each of the new prior art references.

The Invention

The inventors have discovered that mucosal administration of a mixture of a surface antigen of a virus and a second vaccine antigen is effective in generating an enhanced immune response for the prevention or treatment of an infection by either the virus from which the surface antigen is derived, or the agent from which the second vaccine antigen is derived.

Objection to the Drawings

In the final office action issued June 4, 2002, the examiner objected to Figure 5 because the text within the drawing is in the Spanish language. Applicants herewith

submit a replacement Figure 5 in the English language as required by the examiner. Therefore, the objection to the drawings is moot and should be withdrawn.

Objection under 35 U.S.C §132

In the office action issued June 4, 2002, the amendment dated March 12, 2002 was objected to under 35 U.S.C §132 for allegedly introducing new matter into the disclosure. According to the examiner, the amendment adds a "Summary of the Invention" section in which an unsupported concept "a virus-like particle (VLP) comprising a surface antigen" is added.

By the present amendment, applicants have amended the "Summary of the Invention" section by deleting the reference to the surface antigen component of the vaccine as "a virus-like particle (VLP)."

Accordingly, the objection under 35 U.S.C §132 is moot and should be withdrawn.

Rejections under 35 U.S.C §112, second paragraph

In the Office Action claims 11-33 were rejected under 35 U.S.C §112, second paragraph, as being indefinite. According to the examiner, the claims are rendered vague and indefinite because it cannot be determined whether the "mixture" refers to a physical linkage or fusion or whether the antigens are within the same proximity.

During the telephone interview with the undersigned, the examiner stated that the rejection based upon the ambiguity for "mixture" is withdrawn. See also the Interview Summary dated May 14, 2003.

At page 3 of the final Office Action of June 4, 2002, claim 19 is rejected as allegedly unclear. By the present amendment, applicants have cancelled claim 9. Therefore, this rejection is moot and must be withdrawn.

At page 4 of the Office Action the examiner rejected claims 28-30 under 35 U.S.C. §112, second paragraph as allegedly vague and indefinite. Applicants have

cancelled claims 28-30, therefore this rejection under 35 U.S.C. §112, second paragraph is moot and must be withdrawn.

Rejections under 35 U.S.C §112, first paragraph

At page 4, the examiner rejected claims 11-33 under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventor(s) had possession of the invention at the time the application was filed.

Specifically, the examiner alleges that the nature of the mixture and the mixing process is unclear. During the telephone interview with the undersigned, the examiner stated that the rejection based upon the lack of written description for "mixture" language in the claims is withdrawn. See also the Interview Summary dated May 14, 2003.

At page 5, the examiner rejected claim 11-33 under 35 U.S.C. §112, first paragraph allegedly for containing subject matter not described in the specification. Specifically, the examiner rejected claim 11-33 for reciting the phrase "a virus-line particle (VLP) comprising a surface antigen from a virus."

Applicants have cancelled claim 11 and claim 15 *et seq.* has been amended and does not refer to a virus-like particle (VLP). Similarly, the dependent claims do not refer to a virus-like particle (VLP). Therefore, this rejection should be withdrawn.

At pages 5-9, the examiner rejected claims 15-19, 22-27, and 31-33 under 35 U.S.C. §112, first paragraph allegedly for lack of enablement. According to the examiner, it cannot be determined whether the claims are directed to treating and preventing both hepatitis B and the infectious disease caused by the agent from which the vaccine antigen is derived (such as HPV or HCV) or just hepatitis B (HBV).

Applicants assert that one of ordinary skill in the art would clearly understand that the vaccines of the present invention are directed to prevention and therapy of hepatitis B disease through an anti-hepatitis B immune response (see page 5, line 31

of the specification) and also prevention and therapy of the disease caused by the agent from which the second vaccine antigen is derived (see page 7, lines 8-13). Thus, it is clear that the claims are directed to treating and preventing both hepatitis B and the infectious disease caused by the agent from which the second vaccine antigen is derived (such as HPV or HCV). Therefore, applicants respectfully request that this rejection be withdrawn.

In addition, the examiner again alleges that the term "mixture" is not clear. During the May 13, 2003 telephone interview, the examiner stated that the rejection based upon lack of written description for "mixture" language is withdrawn. See also the Interview Summary dated May 14, 2003.

Claims 22-37 were also rejected under 35 U.S.C. §112, first paragraph allegedly for lack of written description and for claiming new matter. The claims recite that the instant vaccine formulation is a therapeutic or a preventative vaccine against HBV, HPV, and HCV, respectively. According to the examiner, the original claims and the disclosure on page 5, lines 29-31 provide support for vaccine compositions, however there is no support for the intended use of the vaccine or the instant formulations for preventing or treating certain types of virus infections.

Applicants respectfully disagree. The specification discloses *inter alia* on page 6, lines 10-31, that the vaccine formulation containing HBsAg and HBcAg antigen elicit a wide spectrum of immune response generated by HBcAg which is an important antigen in anti-HBV protection. Therefore, the specification provides support for preventing virus infections.

Furthermore, the specification also discloses the vaccine formulation of the present invention makes it an ideal formulation for therapeutic use. Therefore, the specification provides support for treating virus infections.

Moreover, the specification discloses on page 7, lines 8-16 that the vaccine formulations (for preventive and therapeutic use) can contain HBsAg and other nucleocapsid antigens. Such nucleocapsid antigens are demonstrated in Example 4 of

the specification, and include HPV and HCV. The specification further discloses on page 7, line13 that a synergic effect was observed in the response generated for both the HbsAg antigen and the nucleocapsid antigen. Thus, applicants respectfully request that this rejection by withdrawn.

Starting at page 6 of the Office Action, claims 15-19, 22-27 and 31-33 were rejected under §112. The examiner alleges that the specification fails to teach how to identify a mixture, fusion or complex of HBsAg and any viral nucleocapsid that would satisfy the intended vaccine function. The examiner summarizes the rejection by stating that it would require undue experimentation to practice the claims due to the alleged ambiguity of the claims; the scope of the claims to preventing and treating any infection with the vaccine composition; the lack of guidance as to the CTL responses or the persistence of the antibody responses; the lack of an appropriate animal model or data demonstrating prevention or treatment by vaccine administration; and the lack or predictability in the vaccine art.

Applicants respectfully disagree with the examiner's assertion that it would require undue experimentation to practice that claimed invention. A declaration under 37 C.F.R. §1.132 of Dr. Jules César Aguilar Rubido, a co-inventor named in the present application, is submitted herewith. In the declaration, Dr. Rubido states at paragraph 7 that the results of administration of a mixture of HBsAg and HBcAg show an improved cellular response as compared with HBsAg alone. Further Dr. Rubido states that these results are consistent with the higher IgG2a responses for HBsAg mixed with HBcAg compared with the control HBsAg alone.

At paragraph 8, Dr. Rubido states that the HBsAg plus HBcAg mixture also induces a better lymphoproliferative response than the control HBsAg alone and that the strong lymphoproliferative response correlates with a better course of infection by the HBV.

Moreover, at paragraph 9, Dr. Rubido states that the mixture of HBsAg and HBcAg potentiates the immune response against both antigens, evidencing the synergistic interaction between both antigens. Dr. Rubido states that similar results

are also seen with HBsAG plus other nucleocapsid antigens (paragraph 10) as compared with the control HBsAg alone (paragraph 11).

At paragraphs 12 and 13, Dr. Rubido states that the experiments reported in Example 2 of Exhibit 1 show an enhancing capacity and new properties after nasal administration of the new formulations of HBsAg and virus-like particles (VLPs) as compared with the VLP alone in a transgenic mouse model. Immune tolerance against HBsAg in transgenic mice was shown to be abrogated by administration of the claimed HBsAg plus HBcAg formulation and correlated with the disappearance of HBsAg from the blood. Again, Dr. Rubido states at paragraph 13 citing Table 1, this is in contrast to the result obtained with the commercial HbsAg vaccine, Engrix B adsorbed onto alum salts or alone, administered intraperitoneally.

As stated by Dr. Rubido in the attached declaration at paragraphs 14-16, the chimpanzee is not the only suitable animal model for HBV infection in humans. The immunized mouse model and transgenic mouse model are sufficient to support the initiation of human trials of the claimed mixtures and therefore are *prima facie* adequate models for HBV infection of humans.

As demonstrated by the examples disclosed in the present specification and as explained by Dr. Rubido in the attached declaration, one of ordinary skill in the art of vaccine research and development would clearly understand that the results disclosed would have led to a reasonable expectation of success of the claimed vaccine mixtures in inducing an enhanced immune response to the antigen components of the mixture as compared to the single antigens alone.

For all these reasons, Applicants assert that it would not require undue experimentation to practice the claimed invention and that the pending claims are fully supported in their broadest scope by the specification as filed which teaches that a specific immune response to both the first vaccine antigen and to the second vaccine antigen are generated in the mouse model and that the vaccine antigen exert a synergistic effect on the immune response to each other. Because such data are generally accepted by those of skill in the art as a reasonable basis for initiation of

clinical trials, applicants assert that the burden of proof of demonstrating a reasonable expectation of success has been met.

Therefore, the rejection of claims 15-19, 22-27, and 31-33 under 35 U.S.C. §112, first paragraph should be withdrawn.

Rejection under 35 U.S.C. §102(a)

Initially, in the Office Action of June 4, 2002, the examiner rejected claims 11-14, 20, 21, 28-30 under 35 U.S.C. §102(a) as allegedly anticipated by Balmelli et al. (J. Virol. 72(10): 8220-8229. However, this rejection was not explained in the Office Action and was subsequently withdrawn in the Interview Summary issued June 18, 2002. Therefore this rejection will not be considered further.

Rejection under 35 U.S.C. 103(a)

At page 9 the examiner rejected claims 11-14, 20, 21, 28-30 under 35 U.S.C. §103(a) as allegedly unpatentable over Lowy et al., US Patent No. 5,618,536 and Rose et al., US Patent No. 6,153,201.

Claims 11-14 and 28-30 have been canceled. Therefore, the rejection of claims 11-14 and 28-30 under 35 U.S.C. §103(a) as allegedly unpatentable over Lowy et al. (supra) and Rose et al. are moot and should be withdrawn, which action is earnestly solicited. Furthermore, claims 20 and 21 have been amended to depend from claim 15. Thus, claims 20 and 21 recite a vaccine formulation comprising a surface antigen of Hepatitis B virus (HBsAg) and a second vaccine antigen which is an antigen of a viral nucleocapsid. Nowhere in Lowy et al. or Rose et al. is there any suggestion of the use of a surface antigen of Hepatitis B virus (HBsAg) in a mixture with an antigen of a viral nucleocapsid. Therefore, claims 20 and 21 are not obvious over Lowy et al. and Rose et al. and this rejection of claims 20 and 21 should also be withdrawn.

New Prior Art References

During the personal interview of February 26, 2003 between Examiners Foley and Housel and Dr. Anilionis, it is the understanding of the undersigned that the examiners expressed their belief that bivalent vaccines, including HBcAg were known in the prior art. It is also the undersigned's understanding that the examiners suggested that claims to trivalent vaccines including HBcAg might be allowable.

On this basis, applicants conducted a search of the National Library of Medicine files at http://www4.ncbi.nlm.nih.gov/PubMed/ and also in the United States Patent and Trademark Office database of issued patents at www.uspto.gov. Applicants submit concurrently herewith in an Information Disclosure Statement and PTO form 1449 the result of our search, specifically U.S. Patent Nos. 4,547,368; 6,126,938; 6,153,392; 6,261,765 B1; and 6,436,402 B1.

During the May 13, 2003 telephone interview between the undersigned and Examiner Foley, applicants informed Examiner Foley of the applicants prior art search concerning bivalent vaccines. Applicants informed Examiner Foley of the disclosure of U.S. Patent No. 4,547,368 (the '368 patent). Specifically, that the '368 patent discloses a vaccine containing HBcAg and HBsAg (see column 1, lines 37-40). However, applicants stated that the '368 patent only discloses compositions for subcutaneous or intramuscular administration (see column 2, lines 60-62). There is no disclosure or suggestion in the '368 patent for a vaccine composition for mucosal administration.

In contrast, the present invention is directed to vaccine formulations for mucosal administration. Examiner Foley stated that she would review the '368 patent.

Applicants wish to point out that the use of vaccine formulation for mucosal administration has several advantages. First, it is widely known that immunization by systemic administration is not sufficient in treating pathogens that typically establish in the mucosa. Mucosal administration induces an immune response in mucosa as well as in sera. A more effective immune response is achieved due to the recruitment

of the mucosal immune system. The mucosal immune response is important for infections which can be, for example, sexually transmitted, such as HBV. Parenteral administration (e.g., subcutaneous or intramuscular) typically does not generate a mucosal immune response.

Another advantage is the lack of need for the use of syringes. Therefore, the added cost of vaccines, as well as the side effects and safety problems, associated with injection of vaccines can be avoided.

For the above reasons, applicants believe the '368 patent is not material to the present invention.

During the telephone interview, applicants also advised Examiner Foley that there were several additional references that applicants wanted to make part of the file record. Applicants informed Examiner Foley that they would provide a synopsis of each of the other references in the Preliminary Amendment filed with the RCE. The examiner was agreeable to our suggestion. A synopsis of the references follows:

U.S. Patent No. 6,126,938 to Guy et al. discloses a method and kit for generating a mucosal immune response against an antigen by implementing an immunization protocol comprising administering three immune inducing agents (e.g., antigens) by several routes (see column 3, lines 64-67). The administration routes disclosed include systemic administration and mucosal administration (see column 4, line 50 to column 5, line 7). Guy et al. further discloses that the immune response inducing agents are administered sequentially or simultaneously (i.e., on the same day) (see column 6, line 52 to column 7, line 2).

U.S. Patent No. 6,153,392 to Liao et al. discloses a composition capable of eliciting an immune response in an animal. The composition contains HBcAg complexed with a protein. According to the specification, the term "complexed" means the formation of a dimer or multimer between the HBcAg and one or more proteins (see column 10, lines 8-10). The protein is selected from the group consisting of a serum albumin and a core-like antigen-adjacent protein of a positive

stranded RNA virus (see column 5, lines 11-18). The positive stranded RNA virus is selected from the group consisting of Togaviridae, Coronaviridae, Retroviridae, Picornaviridae, Calciviridae and Flaviviridae (see column 10, lines 56-60). The HBcAg disclosed in Liao et al. is a protein (see column 1, line 60 and column 6, lines 27-29).

U.S. Patent 6,261,765 B1 to McCarthy et al. discloses a composition containing virus-like particles which have encapsulated therein desired moieties. (see column 13, lines27-30). The moieties that can be encapsulated in the virus-like particles include nucleic acid sequences, radionuclides, hormones, peptides, antiviral agents, antitumor agents, cell growth modulating agents, cell growth inhibitors, cytokines, antigens, and toxins (see column 14, lines 5-9).

U.S. Patent 6,436,402 B1 to Zhao et al. discloses a method for making human papilloma virus (HPV) virus-like particles. The virus-like particles express the L1 or L1 and L2 proteins of HPV. Zhao et. al. further discloses a method of inducing an immune response by administering a vaccine composition containing the virus-like particles.

None of the above prior art references disclose a vaccine formulation containing a **mixture** of a first vaccine antigen which is HBsAg and a second vaccine antigen which is a viral nucleocapsid (e.g., HBcAg). The viral nucleocapsid contains both proteins and nucleic acids.

Accordingly, applicants believe these references are not material to the present invention.

During the telephone interview of May 13, 2003 between the undersigned and the examiner, applicants inquired about the new matter rejection cited in the Advisory Action dated January 28, 2003. Specifically, the Advisory Action stated that the adjuvanting effect of the second vaccine antigen is new matter.

Examiner Foley indicated, during the telephone interview of May 13, 2003, that the new matter rejection in the Advisory Action was probably not appropriate. In

the Interview Summary dated May 14, 2003, the examiner stated that the issue of new matter discussed in the Advisory Action is moot.

Support for new claims 38-41 can be found in the specification as originally filed, *inter alia,* in Example 4 and Figure 4.

For the reasons stated above, allowance of pending claims 15-18, 21-27, and 38-41 is earnestly requested. If the examiner has any questions regarding this amendment, the examiner is respectfully requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,

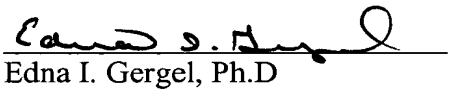

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EXHIBIT 1

Example 1

The interaction of a nucleocapsid antigen (HBcAg) and the HBsAg induced an improved response against both antigens and also against HBcAg evidencing a synergistic response at the cellular level.

A- Study of the capacity to induce gamma IFN secreting cells by ELISPOT assay.

The cellular immune response generated by the nasal formulation of HBsAg and HBcAg, groups of eight weeks old 8 to 10 female balb/c mice were immunised three times at doses of 5μg of HBsAg per mice. Innoculations were carried out times every 2 weeks with the formulations presented in table 1. The immune response was determined two weeks after the last administration.

The ELISPOT assay was performed as described, briefly, lymphocytes from spleens were stimulated with the peptide $HBsAg_{(28-39)}$ presented by the murine tumor cells p815 that were used as antigen presenting cells. Spleen cells were isolated after surgical excission, treatment with ammonium chloride as erythrocyte lysis solution and then washed three times.

After washing steps, the cells were counted and distributed at $2x10^6$ cells per milliliter in 10 mL RPMI fetal calf serum in $25cm^2$ flasks (Nunc), and stimulated with 10 μg/mL of peptide $HBsAg_{(28-39)}$. After culturing during four days, in CO_2 5%, half of the total medium was substituted and new media containing $2x10^4$ U/mL of IL2 was added. On day seven, cells were collected and counted. Subsequently, 10^4 and $5x10^4$ cells per well were added to 10^5 p815 cells, previously pulsed with the peptide for one hour. Stimulation with 2μg per well concanavalin A was used as a positive control of the assay. Every group was controlled by the same number of wells incubated with non-pulsed p815 cells as a negative control.

As a result of this experiment, the highest response was obtained in the group immunized with the nasal formulation HBsAg + HBcAg (fig 1). The results obtained demonstrated the capacity of the nasal route to induce strong gamma-interferon (γIFN) secretion in spleen cells, inducing even better responses than the control group of parenterally administered alum-based

vaccine. Also the enhancement of the immune response against HBsAg after coadministration of HBsAg and HBcAg was shown, evidencing the capacity of HBcAg to improve the cellular response against HBsAg administered alone in PBS. These results have therapeutic significance due to the involvement of T-cell gamma interferon secretion in HBV clearance. These results are consistent with the higher IgG2a response for HBsAg mixed with HBcAg compared with the above mentioned controls.

Fig 1. Results of the ELISPOT experiment to determine the capacity of gamma IFN secretion by spleen lymphocytes. The results of the experiment are expressed in cells per million.

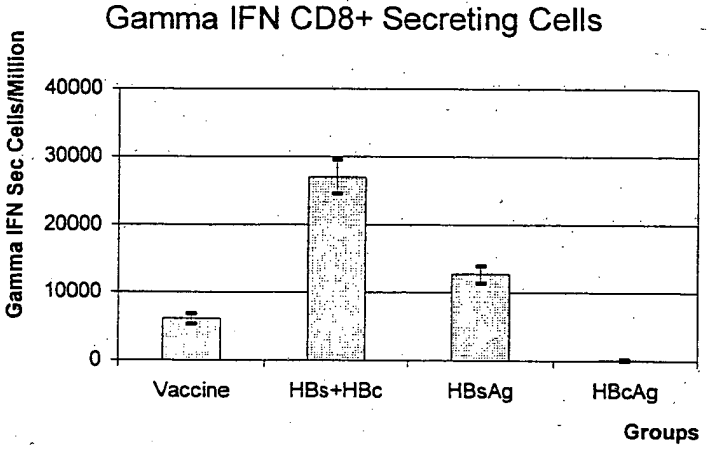


Table 1

Groups	route
G1- 5μg HBsAg / alum	IM
G2- 5μg HBsAg / 5μg HBcAg	IN
G3- 5μg HBsAg / PBS 1X (*)	IN
G4- 5μg HBcAg / PBS 1X (*)	IN
G5- Non Immunised group	

B- Study of the lymphoproliferative response in spleen cells by LPA assay.

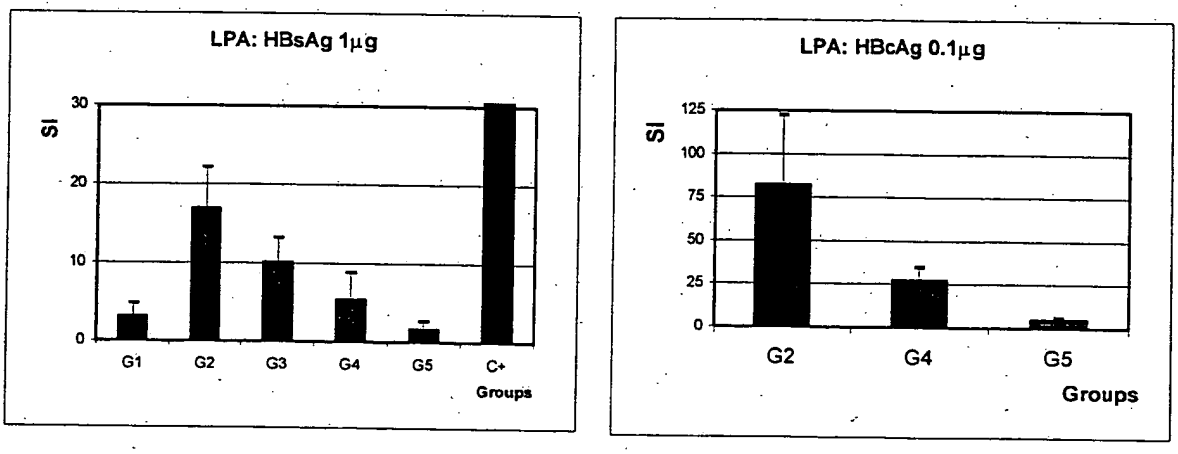
Cells of the animals immunized with the formulation HBsAg + HBcAg described above, and respective controls, were incubated in 5% CO_2 at 37 degrees Celsius in culture plates of 96 wells, in presence of HBsAg in concentrations of 1 and 0.1 μg/mL, during 4 days. Then, cells were pulsed with H^3 thymidine at 1 μCi per well. Cells were cultured by 12 hours and then were harvested and counted with a scintillation counter at 1-minute intervals per assay per well.

The results corresponding to the stimulation index (SI) of the lymphoproliferative response in the immunization conditions previously described are presented in the figure 2A.

These results demonstrated the superiority of the nasal formulation HBsAg + HBcAg in terms of lymphoproliferative response specific for HBsAg.

This results also has significance in the design of therapeutic strategies taking into consideration that a strong lymphoproliferative responses correlates with a better course of the infection by HBV, suggesting a potential improvement of the immune response after therapeutic administration of these major HBV antigens in the HBsAg + HBcAg formulation compared to the alum based vaccine. Also, at the cellular level, the combination of both antigens potentiates the immune response against both antigens, evidencing the synergistic interaction between both antigens (figure 2B). The other formulations of HBsAg and nucleocapsid antigens also had a similar behavior (figure 2B).

Fig 2. Results of the LPA assay. The results of the LPA experiment represent the stimulation index of wells incubated with (A) HBsAg 1μg/mL and (B) HBcAg 0.1μg/mL and their standard deviation.



Example 2

Overcoming the tolerance state in transgenic mice.

An immunization schedule with the formulations containing HBsAg and different nucleocapsid antigens (HBcAg, HPV VLPs, HCcAg and GAG) was carried out in six groups of five BALB/C transgenic mice carrying the HBV antigens with an average of 15 μg/mL in sera. These mice have demonstrated to be tolerant to the HBsAg and HBeAg at the cellular and humoral levels.

The immune tolerance against HBsAg in transgenic mice was abrogated by the administration of 5 doses of the HBcAg + HBsAg formulation, and correlated with the disappearance of the HBsAg from the blood. The other formulations based in the mixture of HBsAg and other VLPs: HBsAg + HCcAg or HBsAg + VLP of HPV, also generated the same effect in this animal model. This result was in contrast to the result obtained with the commercial vaccine, Engerix B, where the HBsAg is absorbed to alum salts and inoculated parenterally by the intraperitoneal route or compared to the use of HBsAg alone. The results are summarized in table 1.

The result described in this example clearly demonstrated the enhancement of the immune response due to the new formulations for nasal administration of HBsAg in solution, coadministered with other VLP, showing an enhancing capacity and generating new properties to the resulting immune response. The resulting response against all the VLPs joined to HBsAg was strongly enhanced as compared to the VLP alone. This demonstrates the crossed effect in enhancing capacity of these antigens. This result is consistent with the immune response observed in normal mice.

Table 1. HBsAg secreting HBV tg mice at day 100 after five doses of 5 mcg of both antigens.
* mice clearing the HBsAg from blood developed strong lymphoproliferative (LPA) response.

Antigen Formulation	HBsAg in sera	LPA anti HBsAg over 5 of Stim.Index
HBsAg + HBcAg (A)	0/5	5/5
+ HCcAg	0/5	5/5
+ VLP HPV	1/5	4/5
+ GAG	0/5	5/5
HBsAg alone	4/5	1/5*
Engerix B	5/5	0/5
Non treated tg mice	5/5	0/5
Non tg mice + Engerix B	0/5	5/5

The levels of IgG titers specific for HbsAg developed by HBsAg transgenic (Tg) mice were statistically superior ($p<0.05$) to those generated in Tg mice after the administration of the commercial vaccine. As shown in table 1, the cellular response was higher for those mice clearing the HBsAg from the blood as was also evidenced in the mouse that cleared the HBsAg in the group immunized with HBsAg alone.

It is important to point out that the effect observed for groups containing HBsAg and a viral nucleocapsid were not observed for the groups immunized with the respective nucleocapsid alone. This excludes the possibility of a non-specific response caused by nucleocapsid antigens. The already explained superiority in mucosal and cellular responses was also obtained for transgenic mice, in those groups immunized with the formulations based in HBsAg and different

nucleocapsid antigens compared to the group of Tg mice immunized with the commercial vaccine as a further support of the therapeutic use of the formulations of HBsAg and nucleocapsid antigens in the treatment of the HBV chronic infection.

Example 3

Tolerance induced by injection and oral feeding

Preliminary results to explore the subversion of tolerance state to HCV core protein, HPV E1-E2 protein and the HIV gag protein in transgenic mice expressing the corresponding antigen in sera have evidenced the capacity of this kind of formulations to overcome the normal state of non responsiveness to the antigens expressed found in those Tg mice at the cellular levels. In the case of these antigens, our experiments have shown the abrogation of tolerance induced by peritoneal injection of high doses and the feeding of a low amount of antigens for more than a month.

These specific models where the tolerance state was obtained by the injection of high amounts of the corresponding antigens along with antigen feeding, demonstrated the capacity of the described formulations nasally administered to subvert the tolerant state to the particular antigens. Conversely, it was impossible to change this state in mice injected parenterally with the same antigens in alum. The immune response was controlled by the induction of proliferative activity in individual mice to the antigens used to tolerize them. All mice treated with the nasal formulations of HbsAg and the homologous antigen (the antigen used to tolerize) induced a stimulation index superior to five (5), while control mice immunized with the homologous antigen in alum did not proliferate sufficiently to be considered clearly positive.

This kind of animal model and treatments are also used in the simulation of chronic diseases like autoimmune processes. Our preliminary results with the model based in transgenic mice to simulate the tolerance induction during chronic diseases point to similar results to those obtained in example 2.

Fifth Schedule

1-10µg HCV NC/ PBS 1X IN

2-5µg HBsAg/ PBS 1X IN

3-10µg HBsAg/ 10µg HCV NC / PBS 1X IN

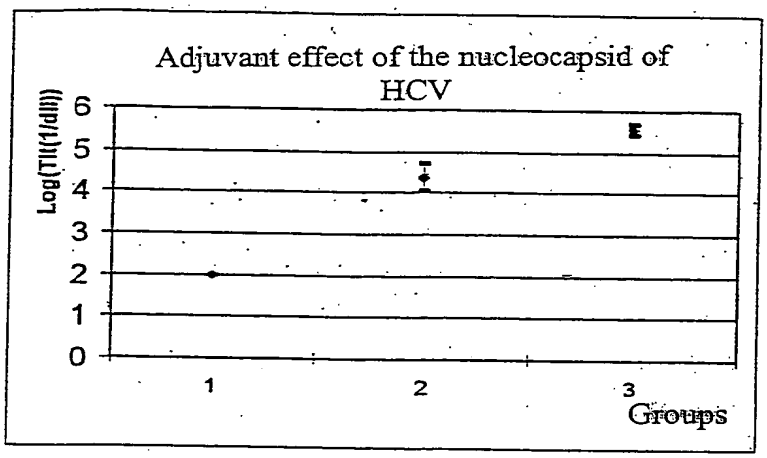


Fig. 5

#19 Decl. 6.9.03

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	J.C. Aguilar Rubido, et al.	Examiner:	Shanon A. Foley
Serial No.:	09/857,402	Group Art Unit:	1648
Confirmation No.:	3056	Docket:	976-11 PCT/US
Filed:	June 1, 2001	Dated:	December 2, 2002
For:	PREPARATIONS CONTAINING VIRUS-LIKE PARTICLES AS IMMUNOPOTENTIATORS ADMINISTERED THROUGH THE MUCOSA		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

Sir,

I, Julio César Aguilar Rubido, of Havana, Cuba, do hereby declare and state as follows:

1. I am a co-inventor named in U.S. patent application serial number 09/857,402 filed on June 1, 2001.

2. I hold a BSc degree in Biochemistry from Havana University.

3. I am employed as researcher by The Center for Genetic Engineering and Biotechnology, Havana, Cuba, the assignee of the above-referenced patent application.

4. I have worked in the field of vaccine and immunology research for 7 years.

5. The experiments described in Exhibit 1 were done by me or by persons directly under my supervision and control.

6. In the experiment entitled "A-Study of the capacity to induce gamma IFN (IFNγ) secreting cells by ELISPOT assay"described at page 1 of Exhibit 1, the highest response

was obtained in the group immunized with the nasal formulation HBsAg+HBcAg (fig 1). The results obtained demonstrated the capacity of the nasal route to induce strong gamma-interferon (IFNγ) secretion in spleen cells, inducing even better responses than the control group of parenterally administered alum-based vaccine.

7. Further, the enhancement of the immune response against HBsAg after coadministration of HBsAg and HBcAg was shown, evidencing the capacity of HBcAg to improve the cellular response against HBsAg administered alone in PBS. These results have therapeutic significance due to the involvement of IFNγ secretion by T-cells in HBV clearance. These results are consistent with the higher IgG2a response for HBsAg mixed with HBcAg compared with the above mentioned controls.

8. The results of experiments described at pages 2-3 of Exhibit 1 entitled "B-Study of the lymphoproliferative response of spleen cells by LPA" demonstrated the superiority of the nasal formulation HBsAg+HBcAg in terms of lymphoproliferative response specific for HBsAg. These results are highly significant for the design of therapeutic strategies taking into consideration the fact that a strong lymphoproliferative response correlates with a better course of the infection by HBV. These results show an improvement of the immune response after therapeutic administration of these major HBV antigens in the HBsAg + HBcAg formulation compared to the alum based vaccine.

9. Furthermore, at the cellular level, the combination of both antigens potentiates the immune response against both antigens, evidencing the synergistic interaction between both antigens (figure 2B). The other formulations of HBsAg and nucleocapsid antigens also had a similar behavior (also shown in figure 2B).

10. The immune tolerance against HBsAg in transgenic mice was abrogated by the administration of five (5) doses of the HBcAg+HBsAg formulation, and correlated with the disappearance of the HBsAg from the blood. The other formulations based in the

mixture of HBsAg and other VLPs: HBsAg+HCcAg or HBsAg+VLP of HPV, also generated the same effect in this animal model.

11. These results described in paragraph 10 was in contrast to the result obtained with the commercial vaccine, Engerix B, where the HBsAg is absorbed to alum salts and inoculated parenterally by the intraperitoneal route or compared to the use of HBsAg alone. The results are summarized in table 1 of Exhibit 1.

12. The results described in Example 2 of Exhibit 1 clearly demonstrate the enhancement of the immune response due to the new formulations for nasal administration of HBsAg in solution, coadministered with a VLP, showing an enhancing capacity and generating new properties to the resulting immune response. The resulting response against all the VLPs when mixed with HBsAg was strongly enhanced as compared to the VLP alone. This demonstrates the crossed effect in enhancing capacity of these antigens. This result in transgenic mice is consistent with the immune response observed in normal mice.

13. The immune tolerance against HBsAg in transgenic mice was abrogated by the administration of five (5) doses of the HBcAg+HBsAg formulation, and correlated with the disappearance of the HBsAg from the blood. The other formulations of mixtures of HBsAg and other VLPs: HBsAg+HCcAg or HBsAg+VLP of HPV, also generated the same effect in this animal model. This result was in contrast to the result obtained with the commercial vaccine, Engerix B, where the HBsAg is absorbed to alum salts and inoculated parenterally by the intraperitoneal route or compared to the use of HBsAg alone. The results are summarized in table 1.

14. In my professional opinion the chimpanzee is not the only model for human viral disease due to HCV or any other chronic disease. Normal and transgenic mice have are useful models to test the goal of therapeutic immunization before administration to humans.

15. One of the main characteristics of the human chronic disease is their specie-specificity. Some infections do not develop all the phases found in humans and in some other cases,

the disease is not reproduced in the same intensity as in humans. The above-described experiments show that it is possible to overcome the tolerance with the claimed formulations.

16. Our studies in humans are now in the first stages for prevention and treatment of chronic hepatitis B and are based in the already explained results in our animal models, hence we don't need to use chimps.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Dated: 4/12/2002

Signed: _____
Julio César Aguilar Rubido